Lab Notebook: July 2, 2016
Finding Stadard Curve of Perchlorate

Group: Perchlorate Team

Standard curves are used to determine the concentration of substance. One way to find the standard curve is to use a spectrophotometer or nanodrop. A spectrophotometer measures the absorbance of the substance. A standard curve is created through the relationship between the known concentration and the absorbance of the substance.

Procedure:

Preparing Sodium Perchlorate Stock Solutions:

1. Measure out 0.06122 g, 0.12244 g, 0.24488 g, 0.48976 g, 0.97952 g, and 1.95904 g of solid sodium perchlorate.
2. Dissolve sodium perchlorate into 50mL of milliq water to create a 0.1M, 0.2M, 0.4M, 0.8M, 1.6M, and 3.2M stock solution, respectively.

Measuring Absorbance of Perchlorate

1. Blank nanodrop with 2uL of milliq water.
2. Measure absorbance of each concentration.

Molar Concentrations of Sodium Perchlorate in Water

	Trials					
Molar Concentration (M)	1		2		3	
	A260	A280	A260	A280	A260	A280
0.1	-0.016	-0.050	-0.015	-0.074	-0.037	-0.065
0.2	-0.071	-0.137	-0.100	-0.163	-0.049	-0.064
0.4	-0.66	-0.110	-0.95	-0.187	-0.026	-0.043
0.8	-0.112	-0.175	-0.155	-0.221	0.009	-0.024
1.6	-0.118	-0.216	0.107	-0.203	0.050	0.029
3.2	-0.172	-0.268	-0.182	-0.273	0.012	0.002

Summary:

The A260 and A280 should not be negative numbers; nanodrop is insufficient in reading the absorbance for perchlorate so a different method must be used to find unknown concentration.

Lab Notebook: July 20, 2016
Cloning of Cld-sp G-Block

Group: Quantitative Bio

The lab aims for the successful cloning of our modified Cld-sp and Cld+sp g-block. The modified minus g-block incorporates the original DNA sequence coding the mature protein structure of the cld enzyme from *Ideonella dechloratans* with the introduction of a silent mutation to prevent digestion at an internal BsaI recognition site. XbaI and BsaI restriction sites are added upstream the start codon, while BsaI, SpeI, NotI and PstI restriction sites are included after two consecutive stop codons. A sequence coding for a polyhistidine-tag is also included downstream of the mature coding sequence for protein purification. The modified plus g-block, on the other hand, has an additional sequence coding for the maltose-binding protein (malE) signal sequence. Fusing the N-terminus with the localization peptide would transport the recombinant protein to the periplasmic space, where we expect to have the greatest concentration of the chlorite ion substrate.

Note: Cld+sp gene has a Mal E signal peptide.

Procedure:

1. Labeled the 0.2 mL PCR tube with “Cld-”.
2. Pipetted 1 µL of CPB-38-441 plasmid, 4 µL of Milli-Q water, and 5 µL of modified Cld-sp G-block to the tube. Mixed the contents of the tube.
3. Added 1.1 µL of 10x NEB cutsmart buffer and 0.5 µL of BsaI-HF. Mixed gently, and pulsed down in a centrifuge.

4. Incubated at 37°C for 1 hour and 5 minutes.
   In: 11:15 am *Out: 12:20 pm*

5. BsaI-HF is heat killed in a thermocycler set at 65°C for 20 minutes.
6. The tube is pulsed down in a centrifuge.
7. 8 µL of Milli-Q water, 2 µL of T4 DNA ligase buffer and 0.5 µL of DNA ligase is pipetted to the tube. Mixed gently and pulsed with a centrifuge.
8. Incubated at room temperature overnight.

Summary:

Cld-sp G-block is cloned to a CPB-38-441 plasmid between BsaI restriction sites.

Lab Notebook: July 21, 2016
Transformation of Cld-sp G-Block and Plating

Group: Quantitative Bio

After the cloning of the Cld-sp G-Blocks to CPB-38-441 plasmids, we are transforming uncloned plasmids (as positive control) and our cloned plasmids to competent DH5-alpha *E. coli* cells. Once transformation has been completed, we will plate cells without the plasmid, cells with the uncloned plasmid and cells with the cloned plasmid. Since there is uncertainty about the type of antibiotic resistance gene present in the plasmid backbone, we are plating the cells in kanamycin plates and ampicillin plates.

Procedure:

Transformation of Cloned Plasmids and CPB-38-441 Plasmids:

1. Added 1 µL of the clone expression to a tube of DH5-alpha *E. coli* cells. Labeled this tube with "Cld-".
2. Added 10 µL of CPB-38-441 plasmid to a tube of DH5-alpha *E. coli* cells. Labeled this tube with "+ control".
3. Labeled a tube that contains only DH5-alpha *E. coli* cells with "No DNA".
4. Placed all three tubes in an ice bucket and let them sit for 30 minutes.
5. Heat shocked the three tubes in a heating bath set at 42°C for 90 seconds.
6. Transferred all three tubes in an ice bucket and let them sit for 5 minutes.
7. Added 1 mL of LB Broth to each tube.
8. Incubated them for approximately 1 hour at 37°C.
   In: 12:18 pm *Out: 1:32 pm*

Plating of DH5-alpha E. coli cells:

1. Pipetted 200 µL from the "No DNA" tube to each of one non-antibiotic plate, one kanamycin plate and one ampicillin plate.
2. Used a spreader to gently spread out the liquid in each plate.
3. Pipetted 200 µL from the "+ control" tube to each of one kanamycin plate and one ampicillin plate.
4. Used a hockey stick to gently spread out the liquid in each plate.
5. Pipetted 200 µL from the "Cld-" tube to each of one kanamycin plate and one ampicillin plate.
6. All plates were placed under the fume hood with lids off until they were dry.
7. All plates were flipped (lid on the bottom) and incubated at 37°C overnight.

Summary:

- A tube of DH5-alpha *E. coli* is our negative control ("No DNA").
- CPB-38-441 plasmids are transformed into DH5-alpha *E. coli* ("+ control").
- CPB-38-441 plasmids cloned with the Cld-sp genes are transformed into DH5-alpha *E. coli* ("Cld-").
- Cells from the "No DNA" tube are plated in agar plates without antibiotic, plates with kanamycin and plates with ampicillin.
- Cells from the "+ control" tube are plated in agar plates with kanamycin and plates with ampicillin.
- Cells from the "Cld-" tube are plated in agar plates with kanamycin and plates with ampicillin.
- All plates are incubated at 37°C overnight.

Lab Notebook: July 25, 2016
Viewing Plates and Incubation of Culture Tubes

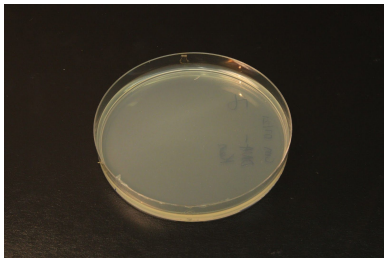
Group: Quantitative Bio

Observations on the plates are recorded. Six white colonies from each of the Cld-sp and Cld+sp kanamycin plates are inoculated into 5 mL LB Broth with kanamycin antibiotic and incubated overnight.

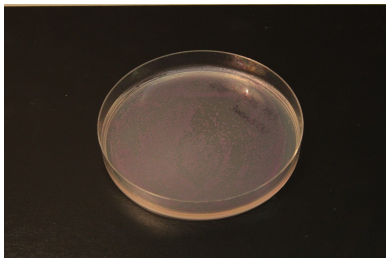
Observation of Colony Growth on Kanamycin Plates

	Antibiotic	**Qualitative**
Negative Control	None	Translucent white film
	Ampicillin	No growth
	Kanamycin	No growth
Positive Control	Ampicillin	No growth
	Kanamycin	Purple colonies
Cld Negative	Ampicillin	No growth
	Kanamycin	White and purple colonies
Cld Positive	Ampicillin	No growth
	Kanamycin	White and purple colonies

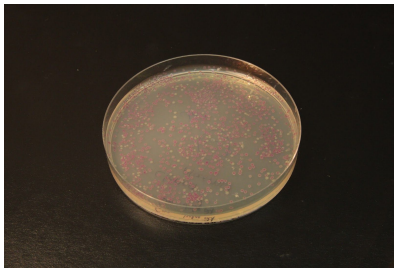
Images:



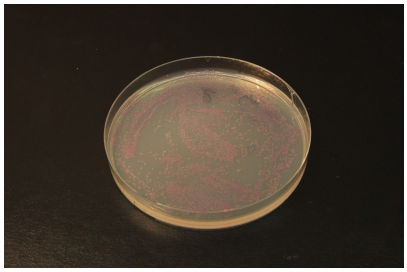
Negative Control



Positive Control



Cld-sp



Cld+sp

Procedure:

1. 100 µL of 1000x kanamycin stock solution is pipetted into 100 mL of LB Broth.
2. Twelve tubes are labeled from 1 to 12 with a subtitle of “Cld-” for the first six tubes and a subtitle of “Cld+” for tubes 7 to 12.
3. 5 mL of LB Broth with kanamycin is pipetted into each of the twelve tubes.
4. Using a P2 micropipette tip, a white colony is scraped from the Cld Negative kanamycin plate and the micropipette tip is submerged into the appropriately labeled tube. The same procedure is used to inoculate the remaining five Cld- tubes.
5. Using a P2 micropipette tip, a white colony is scraped from the Cld Positive kanamycin plate and the micropipette tip is submerged into the appropriately labeled tube. The same procedure is used to inoculate the remaining five Cld+ tubes.
6. All tubes were incubated at 37°C overnight.

Summary:

- No growth is observed on all ampicillin agar plates, as the CPB-38-441 plasmids encode for kanamycin resistance.
- No single colonies are visible on the non-antibiotic agar plate with negative control.The translucent white film on the plate is evident of high levels of bacterial growth.
- On the positive control kanamycin agar plate, only multiple purple colonies are visible. Positive control encodes for the colour expression gene and no cld genes have been ligated.
- White and purple colonies are visible in the Cld Negative and Cld Positive kanamycin agar plates. There is a significantly higher proportion of purple colonies as compared to white colonies in both plates, with the Cld Positive plate having lower number of white colonies.
- Six colonies from each of the Cld Negative and Cld Positive kanamycin agar plate were inoculated into each of the appropriately-labeled culture tubes. They are incubated at 37°C overnight in the shaker.

Lab Notebook: July 26, 2016
Incubated Culture Tubes Result and Redo

Group: Quantitative Bio

After observing inconsistent bacterial growth across all twelve tubes, with some tubes having a lower density of *E. coli* compared to others, we decided to repeat the preparations for overnight cultures. This inconsistency is likely caused by contamination from fully submerging the pipette tips into LB media during inoculation.

Procedure:

1. 100 µL of 1000x kanamycin stock solution is pipetted into 100 mL of LB Broth.
2. Twelve tubes are labeled from 1 to 12 with a subtitle of “Cld-” for the first six tubes and a subtitle of “Cld+” for tubes 7 to 12.
3. 5 mL of LB Broth with kanamycin is pipetted into each of the twelve tubes.
4. Using the needle end of the inoculation loop, a white colony is scraped from the Cld Negative kanamycin plate and vigorously mixed into the appropriately labeled tube. The same procedure is used to inoculate the remaining five Cld- tubes.
5. Using the needle end of the inoculation loop, a white colony is scraped from the Cld Positive kanamycin plate and vigorously mixed into the appropriately labeled tube. The same procedure is used to inoculate the remaining five Cld+ tubes.
6. All tubes were placed at an angle in the shaker and incubated at 37°C overnight.

Summary:

- Inconsistency in bacterial growth in the overnight cultures is likely caused by possible contamination from fully submerging pipette tips into the media.
- Six colonies from each of the Cld Negative and Cld Positive kanamycin agar plate were inoculated into each of the appropriately-labeled culture tubes. They are incubated at 37°C overnight in the shaker.
- Tubes are put at an angle in the incubator shaker to cover more surface area.

Lab Notebook: July 27, 2016
Incubated Culture Tubes Redo for Cld+sp, and Cld-sp Miniprep

Group: Quantitative Bio

Tubes with Cld Positive all contain purple culture caused by an inoculation of these tubes with purple colonies instead of white ones. Preparation for Cld Positive overnight cultures have to be repeated. Cld Negative overnight cultures, on the other hand, exhibited relatively consistent bacterial growth across six tubes. Minipreps for each of the six Cld Negative overnight cultures were done to extract the recombinant plasmid.

Procedure:

Inoculation and Incubation of Overnight Cultures

1. 50 µL of 1000x kanamycin stock solution is pipetted into 50 mL of LB Broth.
2. Six tubes are labeled from 7 to 12 with a subtitle of “Cld+”.
3. 5 mL of LB Broth with kanamycin is pipetted into each of the twelve tubes.
4. Using the needle end of the inoculation loop, a white colony is scraped from the Cld Positive kanamycin plate and vigorously mixed into the appropriately labeled tube. The same procedure is used to inoculate the remaining five Cld+ tubes.
5. All tubes were placed at an angle in the shaker and incubated at 37°C overnight.

Cld-sp Miniprep

1. Label six microcentrifuge tubes from 1 to 6 with a subtitle of “Cld-”.
2. Pipette 1 mL of overnight culture from the culture tubes into their corresponding microcentrifuge tubes and centrifuge at 13 000 rpm for 1 minute. Decant the supernatant. Repeat this step until 3 mL worth of culture has been centrifuged. If needed, pipette out the remaining liquid.
3. Add 250 µL of Buffer P1 into each of the tubes and resuspend the pellet by vortexing.
4. Add 250 µL of Buffer P2 into each of the tubes and invert the tubes 5-6 times.
5. Add 350 µL of Buffer N3 into each of the tubes.
6. Centrifuge all tubes at maximum speed for 10 minutes.
7. Label six QIAprep 2.0 spin column from 1 to 6 with a subtitle of “Cld-”.
8. Pipette a minimum of 800 µL of supernatant from each of the microcentrifuge tubes into their corresponding QIAprep 2.0 spin column.
9. Centrifuge all the columns at maximum speed for 1 minute.
10. Decant flow through.
11. Centrifuge all the columns at maximum speed for 1 minute.
12. Label six microcentrifuge tubes from 1 to 6 with a subtitle of “Cld-”.
13. Transfer columns to their corresponding microcentrifuge tubes.
14. Add 100 µL of Buffer EB and let the solution sit for 1 minute.
15. Centrifuge all the tubes at maximum speed for 1 minute and discard the columns.

NanoDrop

1. Blank the NanoDrop twice with 2 µL of Buffer EB.
2. Measure the 260/280 values and DNA concentration of 2 µL of each of the recombinant plasmid samples twice.

DNA 260/280 Values and Concentrations of Cld-sp Miniprep Samples

Sample	Trial			
	1		2	
	260/280	Concentration (µg/mL)	260/280	Concentration (µg/mL)
1	1.77	88.4	1.80	85.0
2	1.81	84.4	1.80	85.0
3	1.79	87.0	1.81	78.6
4	1.79	83.6	1.79	83.1
5	1.79	77.7	1.77	81.2
6	1.81	76.7	1.79	77.2

Summary:

- Evident from the purple overnight cultures, Cld+sp tubes are contaminated with *E. coli* transformed with the plasmid expressing the chromogenic protein.
- Preparing Cld Positive overnight cultures are repeated.
- Minipreps for each of the six Cld Negative overnight cultures were done to extract the recombinant plasmid.
- The purity of extracted plasmid DNA from Cld Negative overnight cultures is relatively high. All 260/280 values are close to 1.8.
- The concentration of extracted plasmid DNA is relatively high.

Lab Notebook: July 28, 2016
Cld+sp Miniprep and Gel Electrophoresis

Group: Quantitative Bio

Cld Positive overnight culture tubes are ready for miniprep with no purple cultures observed in any tube. Minipreps for each of the six Cld Positive overnight cultures were done to extract the recombinant plasmid. All Cld Negative and Cld Positive plasmid samples were run through a gel to confirm the base pair count of the inserted gene. The plasmid backbone has approximately 4 kilobase pairs, while the chromogenic gene has around 0.7 kilobase pairs. Our inserted Cld Positive and Cld Negative genes each have approximately 1 kilobase pair.

Procedure:

Cld+sp Miniprep

1. Label six microcentrifuge tubes from 1 to 6 with a subtitle of “Cld+”.
2. Pipette 1 mL of overnight culture from the culture tubes into their corresponding microcentrifuge tubes and centrifuge at 13 000 rpm for 1 minute. Decant the supernatant. Repeat this step until 3 mL worth of culture has been centrifuged. If needed, pipette out the remaining liquid.
3. Add 250 µL of Buffer P1 into each of the tubes and resuspend the pellet by vortexing.
4. Add 250 µL of Buffer P2 into each of the tubes and invert the tubes 5-6 times.
5. Add 350 µL of Buffer N3 into each of the tubes.
6. Centrifuge all tubes at maximum speed for 10 minutes.
7. Label six QIAprep 2.0 spin column from 1 to 6 with a subtitle of “Cld+”.
8. Pipette a minimum of 800 µL of supernatant from each of the microcentrifuge tubes into their corresponding QIAprep 2.0 spin column.
9. Centrifuge all the columns at maximum speed for 1 minute.
10. Decant flow through.
11. Centrifuge all the columns at maximum speed for 1 minute.
12. Label six microcentrifuge tubes from 1 to 6 with a subtitle of “Cld+”.
13. Transfer columns to their corresponding microcentrifuge tubes.
14. Add 100 µL of Buffer EB and let the solution sit for 1 minute.
15. Centrifuge all the tubes at maximum speed for 1 minute and discard the columns.

NanoDrop

1. Blank the NanoDrop twice with 2 µL of Buffer EB.
2. Measure the 260/280 values and DNA concentration of 2 µL of each of the recombinant plasmid samples twice.

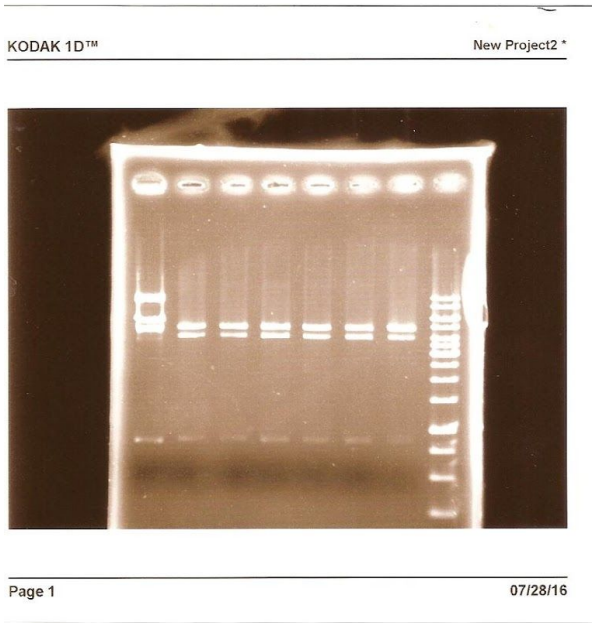
Ligation

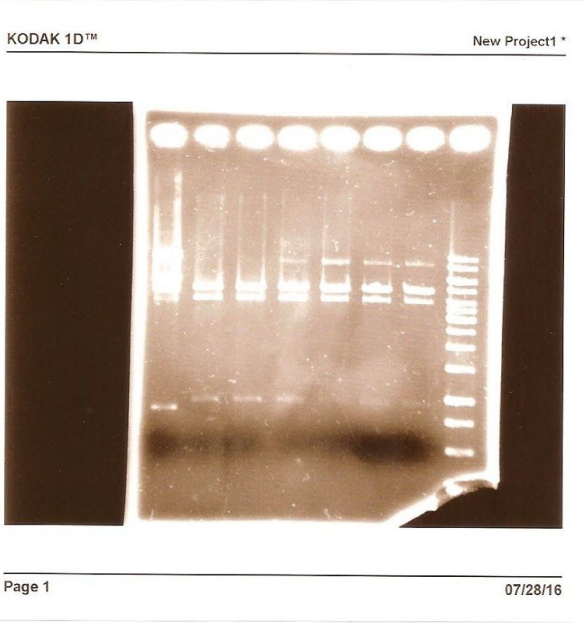
Gel Electrophoresis

DNA 260/280 Values and Concentrations of Cld+sp Miniprep Samples

Sample	Trial			
	1		2	
	260/280	Concentration (µg/mL)	260/280	Concentration (µg/mL)
1	1.78	86.7	1.83	85.4
2	1.79	98.5	1.79	98.6
3	1.83	81.9	1.82	82.9
4	1.82	97.1	1.80	97.3
5	1.82	92.7	1.82	93.9
6	1.82	95.4	1.82	95.4

Images:





First image shows Cld-sp, while the second shows Cld+sp

Summary:

- Minipreps for each of the six Cld Positive overnight cultures were done to extract the recombinant plasmid.
- The purity of extracted plasmid DNA from Cld Positive overnight cultures is relatively high. All 260/280 values are close to 1.8.
- The concentration of extracted plasmid DNA is relatively high.